



SEQUENCE LISTING

<110> TAKAKURA, Hikaru
MORISHITA, Mio
YAMAMOTO, Katsuhiko
MITTA, Masanori
ASADA, Kiyozo
TSUNASAWA, Susumu
KATO, Ikunoshin

<120> ULTRATHERMOSTABLE PROTEASE GENES

<130> TAKAKURA=1A

<140> 09/841,553

<141> 2001-04-24

<150> 08/894,818

<151> 1997-08-29

<150> JP32385/1995

<151> 1995-12-12

<150> JP96/03253

<151> 1996-11-07

<160> 45

<170> PatentIn version 3.2

<210> 1

<211> 659

<212> PRT

<213> Thermococcus celer

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Asn Ala Val Gln Gln Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe
35 40 45

Lys Lys Val Gln Arg Met Asn Trp Asn Gln Glu Val Asp Thr Val Ile
50 55 60

Met Phe Gly Ser Tyr Gly Asp Arg Asp Arg Ala Val Lys Val Leu Arg
65 70 75 80

Leu Met Gly Ala Gln Val Lys Tyr Ser Tyr Lys Ile Ile Pro Ala Val
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Ala Val Lys Ile Lys Ala Arg Asp Leu Leu Leu Ile Ala Gly Met Ile
100 105 110

Asp Thr Gly Tyr Phe Gly Asn Thr Arg Val Ser Gly Ile Lys Phe Ile
115 120 125

Gln Glu Asp Tyr Lys Val Gln Val Asp Asp Ala Thr Ser Val Ser Gln
130 135 140

Ile Gly Ala Asp Thr Val Trp Asn Ser Leu Gly Tyr Asp Gly Ser Gly
145 150 155 160

Val Val Val Ala Ile Val Asp Thr Gly Ile Asp Ala Asn His Pro Asp
165 170 175

Leu Lys Gly Lys Val Ile Gly Trp Tyr Asp Ala Val Asn Gly Arg Ser
180 185 190

Thr Pro Tyr Asp Asp Gln Gly His Gly Thr His Val Ala Gly Ile Val
195 200 205

Ala Gly Thr Gly Ser Val Asn Ser Gln Tyr Ile Gly Val Ala Pro Gly
210 215 220

Ala Lys Leu Val Gly Val Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
225 230 235 240

Val Ser Thr Ile Ile Ala Gly Val Asp Trp Val Val Gln Asn Lys Asp
245 250 255

Lys Tyr Gly Ile Arg Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
260 265 270

Ser Asp Gly Thr Asp Ser Leu Ser Gln Ala Val Asn Asn Ala Trp Asp
275 280 285

Ala Gly Ile Val Val Cys Val Ala Ala Gly Asn Ser Gly Pro Asn Thr
290 295 300

Tyr Thr Val Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
305 310 315 320

Ala Val Asp Ser Asn Asp Asn Ile Ala Ser Phe Ser Ser Arg Gly Pro
325 330 335

Thr Ala Asp Gly Arg Leu Lys Pro Glu Val Val Ala Pro Gly Val Asp
340 345 350

Ile Ile Ala Pro Arg Ala Ser Gly Thr Ser Met Gly Thr Pro Ile Asn
355 360 365

Asp Tyr Tyr Thr Lys Ala Ser Gly Thr Ser Met Ala Thr Pro His Val
370 375 380

Ser Gly Val Gly Ala Leu Ile Leu Gln Ala His Pro Ser Trp Thr Pro
385 390 395 400

Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Ala Pro
405 410 415

Lys Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Val Tyr
420 425 430

Lys Ala Ile Lys Tyr Asp Asp Tyr Ala Lys Leu Thr Phe Thr Gly Ser
435 440 445

Val Ala Asp Lys Gly Ser Ala Thr His Thr Phe Asp Val Ser Gly Ala
450 455 460

Thr Phe Val Thr Ala Thr Leu Tyr Trp Asp Thr Gly Ser Ser Asp Ile
465 470 475 480

Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Glu Val Asp Tyr Ser Tyr
485 490 495

Thr Ala Tyr Tyr Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Ala
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Gly Thr Trp Thr Val Lys Val Val Ser Tyr Lys Gly Ala Ala Asn Tyr
515 520 525

Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Ser Gly Gly Gly
530 535 540

Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro Thr Pro Thr Thr Asp Thr
545 550 555 560

Gln Thr Phe Thr Gly Ser Val Asn Asp Tyr Trp Asp Thr Ser Asp Thr
565 570 575

Phe Thr Met Asn Val Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu
580 585 590

Thr Phe Asp Thr Ser Tyr Asn Asp Leu Asp Leu Tyr Leu Tyr Asp Pro
595 600 605

Asn Gly Asn Leu Val Asp Arg Ser Thr Ser Ser Asn Ser Tyr Glu His
610 615 620

Val Glu Tyr Ala Asn Pro Ala Pro Gly Thr Trp Thr Phe Leu Val Tyr
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Ala Tyr Ser Thr Tyr Gly Trp Ala Asp Tyr Gln Leu Lys Ala Val Val
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Tyr Tyr Gly

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<213> Thermococcus celer

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 <213> *Pyrococcus furiosus*

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 <222> (428)..(428)
 <223> Xaa is Gly or Val

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 Ile Asp Thr Gly Ile Asp Ala Ser His Pro Asp Leu Gln Gly Lys Val
 35 40 45
 Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr Pro Tyr Asp Asp
 50 55 60
 His Gly His Gly Thr His Val Ala Ser Ile Ala Ala Gly Thr Gly Ala
 65 70 75 80
 Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly Ala Lys Leu Ala
 85 90 95
 Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser Ile Ser Thr Ile
 100 105 110
 Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp Lys Tyr Gly Ile
 115 120 125
 Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser Ser Asp Gly Thr
 130 135 140
 Asp Ala Leu Ser Gln Ala Val Asn Ala Ala Trp Asp Ala Gly Leu Val
 145 150 155 160
 Val Val Val Ala Ala Gly Asn Ser Gly Pro Asn Lys Tyr Thr Ile Gly
 165 170 175
 Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly Ala Val Asp Lys
 180 185 190
 Tyr Asp Val Ile Thr Ser Phe Ser Ser Arg Gly Pro Thr Ala Asp Gly
 195 200 205
 Arg Leu Lys Pro Glu Val Val Ala Pro Gly Asn Trp Ile Ile Ala Ala
 210 215 220
 Arg Ala Ser Gly Thr Ser Met Gly Gln Pro Ile Asn Asp Tyr Tyr Thr
 225 230 235 240

Ala Ala Pro Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ile Ala
 245 250 255

Ala Leu Leu Leu Gln Ala His Pro Ser Trp Thr Pro Asp Lys Val Lys
 260 265 270

Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Lys Pro Asp Glu Ile Ala
 275 280 285

Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Ala Tyr Lys Ala Ile Asn
 290 295 300

Tyr Asp Asn Tyr Ala Lys Leu Val Phe Thr Gly Tyr Val Ala Asn Lys
 305 310 315 320

Gly Ser Gln Thr His Gln Phe Val Ile Ser Gly Ala Ser Phe Val Thr
 325 330 335

Ala Thr Leu Tyr Trp Asp Asn Ala Asn Ser Asp Leu Asp Leu Tyr Leu
 340 345 350

Tyr Asp Pro Asn Gly Asn Gln Val Asp Tyr Ser Tyr Thr Ala Tyr Tyr
 355 360 365

Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Asp Gly Thr Trp Thr
 370 375 380

Ile Lys Val Val Ser Tyr Ser Gly Ser Ala Asn Tyr Gln Val Asp Val
 385 390 395 400

Val Ser Asp Gly Ser Leu Ser Gln Pro Gly Ser Ser Pro Ser Pro Gln
 405 410 415

Pro Glu Pro Thr Val Asp Ala Lys Thr Phe Gln Xaa Ser Asp His Tyr
 420 425 430

Tyr Tyr Asp Arg Ser Asp Thr Phe Thr Met Thr Val Asn Ser Gly Ala
 435 440 445

Thr Lys Ile Thr Gly Asp Leu Val Phe Asp Thr Ser Tyr His Asp Leu
 450 455 460

Asp Leu Tyr Leu Tyr Asp Pro Asn Gln Lys Leu Val Asp Arg Ser Glu
465 470 475 480

Ser Pro Asn Ser Tyr Glu His Val Glu Tyr Leu Thr Pro Ala Pro Gly
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500 505 510

Tyr Glu Leu Thr Ala Lys Val Tyr Tyr Gly
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<212> DNA
<213> Pyrococcus furiosus

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<223> n is G or T

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ggcagccaaa ctcaccagtt cgttattagc ggagcttcgt tcgtaactgc cacattatac      1020
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gactactctt acaccgccta ctatggattc gaaaagggtg gttattacaa cccaactgat      1140
ggaacatgga caattaaggt tgtaagctac agcggaagtg caaactatca agtagatgtg      1200
gtaagtgatg gttccctttc acagcctgga agttcaccat ctccacaacc agaaccaaca      1260
gtagacgcaa agacgttcca agnatccgat cactactact atgacaggag cgacaccttt      1320
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<212> PRT
<213> Artificial Sequence

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<400> 5

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Val Val Gly Ser Val Ala Ala Ala Pro Glu Lys Lys Val Glu Gln Val
          20           25           30

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Arg Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg
          35           40           45

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Lys Ile Gln Lys Leu Asn Pro Asn Glu Glu Ile Ser Thr Val Ile Val
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Phe Glu Asn His Arg Glu Lys Glu Ile Ala Val Arg Val Leu Glu Leu
65           70           75           80

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Met Gly Ala Lys Val Arg Tyr Val Tyr His Ile Ile Pro Ala Ile Ala
          85           90           95

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Ala Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly
 100 105 110

Gly Lys Ala Lys Leu Ser Gly Val Arg Phe Ile Gln Glu Asp Tyr Lys
 115 120 125

Val Thr Val Ser Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
 130 135 140

Val Met Ala Thr Tyr Val Trp Asn Leu Gly Tyr Asp Gly Ser Gly Ile
 145 150 155 160

Thr Ile Gly Ile Ile Asp Thr Gly Ile Asp Ala Ser His Pro Asp Leu
 165 170 175

Gln Gly Lys Val Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr
 180 185 190

Pro Tyr Asp Asp His Gly His Gly Thr His Val Ala Ser Ile Ala Ala
 195 200 205

Gly Thr Gly Ala Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly
 210 215 220

Ala Lys Leu Ala Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
 225 230 235 240

Ile Ser Thr Ile Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp
 245 250 255

Lys Tyr Gly Ile Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
 260 265 270

Ser Asp Gly Thr Asp Ser Leu Ser Gln Ala Val Asn Asn Ala Trp Asp
 275 280 285

Ala Gly Ile Val Val Cys Val Ala Ala Gly Asn Ser Gly Pro Asn Thr
 290 295 300

Tyr Thr Val Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
 305 310 315 320

Ala Val Asp Ser Asn Asp Asn Ile Ala Ser Phe Ser Ser Arg Gly Pro

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Ile Ile Ala Pro Arg Ala Ser Gly Thr Ser Met Gly Thr Pro Ile Asn 355 360 365		
Asp Tyr Tyr Thr Lys Ala Ser Gly Thr Ser Met Ala Thr Pro His Val 370 375 380		
Ser Gly Val Gly Ala Leu Ile Leu Gln Ala His Pro Ser Trp Thr Pro 385 390 395 400		
Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Ala Pro 405 410 415		
Lys Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Val Tyr 420 425 430		
Lys Ala Ile Lys Tyr Asp Asp Tyr Ala Lys Leu Thr Phe Thr Gly Ser 435 440 445		
Val Ala Asp Lys Gly Ser Ala Thr His Thr Phe Asp Val Ser Gly Ala 450 455 460		
Thr Phe Val Thr Ala Thr Leu Tyr Trp Asp Thr Gly Ser Ser Asp Ile 465 470 475 480		
Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Glu Val Asp Tyr Ser Tyr 485 490 495		
Thr Ala Tyr Tyr Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Ala 500 505 510		
Gly Thr Trp Thr Val Lys Val Val Ser Tyr Lys Gly Ala Ala Asn Tyr 515 520 525		
Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Ser Gly Gly Gly 530 535 540		
Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro Thr Pro Thr Thr Asp Thr 545 550 555 560		

Gln Thr Phe Thr Gly Ser Val Asn Asp Tyr Trp Asp Thr Ser Asp Thr
565 570 575

Phe Thr Met Asn Val Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu
580 585 590

Thr Phe Asp Thr Ser Tyr Asn Asp Leu Asp Leu Tyr Leu Tyr Asp Pro
595 600 605

Asn Gly Asn Leu Val Asp Arg Ser Thr Ser Ser Asn Ser Tyr Glu His
610 615 620

Val Glu Tyr Ala Asn Pro Ala Pro Gly Thr Trp Thr Phe Leu Val Tyr
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Ala Tyr Ser Thr Tyr Gly Trp Ala Asp Tyr Gln Leu Lys Ala Val Val
645 650 655

Tyr Tyr Gly

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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

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<210> 7
 <211> 4765
 <212> DNA
 <213> *Pyrococcus furiosus*

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 35 40 45

Lys Glu Val Ser Gln Ala Ala Leu Asn Ala Ile Met Lys Gly Gln Pro
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Asn Met Val Leu Ile Ile Lys Thr Lys Glu Gly Lys Leu Glu Glu Ala
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Lys Thr Glu Leu Glu Lys Leu Gly Ala Glu Ile Leu Asp Glu Asn Arg
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Val Leu Asn Met Leu Leu Val Lys Ile Lys Pro Glu Lys Val Lys Glu
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Leu Asn Tyr Ile Ser Ser Leu Glu Lys Ala Trp Leu Asn Arg Glu Val
 115 120 125

Lys Leu Ser Pro Pro Ile Val Glu Lys Asp Val Lys Thr Lys Glu Pro
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Gln Phe Ile Gln Glu Phe Gly Tyr Asp Gly Ser Gly Val Val Val Ala
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Val Leu Asp Thr Gly Val Asp Pro Asn His Pro Phe Leu Ser Ile Thr
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Pro Asp Gly Arg Arg Lys Ile Ile Glu Trp Lys Asp Phe Thr Asp Glu
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Gly Phe Val Asp Thr Ser Phe Ser Phe Ser Lys Val Val Asn Gly Thr
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Leu Ile Ile Asn Thr Thr Phe Gln Val Ala Ser Gly Leu Thr Leu Asn
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Glu Ser Thr Gly Leu Met Glu Tyr Val Val Lys Thr Val Tyr Val Ser
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Asn Val Thr Ile Gly Asn Ile Thr Ser Ala Asn Gly Ile Tyr His Phe
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Tyr Tyr Tyr Gly Pro Leu Asn Tyr Val Leu Ala Glu Ile Asp Pro Asn 340 345 350		
Gly Glu Tyr Ala Val Phe Gly Trp Asp Gly His Gly His Gly Thr His 355 360 365		
Val Ala Gly Thr Val Ala Gly Tyr Asp Ser Asn Asn Asp Ala Trp Asp 370 375 380		
Trp Leu Ser Met Tyr Ser Gly Glu Trp Glu Val Phe Ser Arg Leu Tyr 385 390 395 400		
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Pro Gly Ala Gln Ile Met Ala Ile Arg Val Leu Arg Ser Asp Gly Arg 420 425 430		
Gly Ser Met Trp Asp Ile Ile Glu Gly Met Thr Tyr Ala Ala Thr His 435 440 445		
Gly Ala Asp Val Ile Ser Met Ser Leu Gly Gly Asn Ala Pro Tyr Leu 450 455 460		
Asp Gly Thr Asp Pro Glu Ser Val Ala Val Asp Glu Leu Thr Glu Lys 465 470 475 480		
Tyr Gly Val Val Phe Val Ile Ala Ala Gly Asn Glu Gly Pro Gly Ile 485 490 495		
Asn Ile Val Gly Ser Pro Gly Val Ala Thr Lys Ala Ile Thr Val Gly 500 505 510		

Ala Ala Ala Val Pro Ile Asn Val Gly Val Tyr Val Ser Gln Ala Leu
515 520 525

Gly Tyr Pro Asp Tyr Tyr Gly Phe Tyr Tyr Phe Pro Ala Tyr Thr Asn
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Val Arg Ile Ala Phe Phe Ser Ser Arg Gly Pro Arg Ile Asp Gly Glu
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Ile Lys Pro Asn Val Val Ala Pro Gly Tyr Gly Ile Tyr Ser Ser Leu
565 570 575

Pro Met Trp Ile Gly Gly Ala Asp Phe Met Ser Gly Thr Ser Met Ala
580 585 590

Thr Pro His Val Ser Gly Val Val Ala Leu Leu Ile Ser Gly Ala Lys
595 600 605

Ala Glu Gly Ile Tyr Tyr Asn Pro Asp Ile Ile Lys Lys Val Leu Glu
610 615 620

Ser Gly Ala Thr Trp Leu Glu Gly Asp Pro Tyr Thr Gly Gln Lys Tyr
625 630 635 640

Thr Glu Leu Asp Gln Gly His Gly Leu Val Asn Val Thr Lys Ser Trp
645 650 655

Glu Ile Leu Lys Ala Ile Asn Gly Thr Thr Leu Pro Ile Val Asp His
660 665 670

Trp Ala Asp Lys Ser Tyr Ser Asp Phe Ala Glu Tyr Leu Gly Val Asp
675 680 685

Val Ile Arg Gly Leu Tyr Ala Arg Asn Ser Ile Pro Asp Ile Val Glu
690 695 700

Trp His Ile Lys Tyr Val Gly Asp Thr Glu Tyr Arg Thr Phe Glu Ile
705 710 715 720

Tyr Ala Thr Glu Pro Trp Ile Lys Pro Phe Val Ser Gly Ser Val Ile
725 730 735

Leu Glu Asn Asn Thr Glu Phe Val Leu Arg Val Lys Tyr Asp Val Glu

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Thr	Thr	Pro	Val	Ile	Glu	Asp	Glu	Ile	Leu	Asn	Thr	Ile	Val	Ile	Pro
	770					775					780				
Glu	Lys	Phe	Thr	Pro	Glu	Asn	Asn	Tyr	Thr	Leu	Thr	Trp	Tyr	Asp	Ile
785						790					795				800
Asn	Gly	Pro	Glu	Met	Val	Thr	His	His	Phe	Phe	Thr	Val	Pro	Glu	Gly
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Arg	Pro	Asp	Gly	Met	Phe	Val	Phe	Pro	Tyr	Gln	Leu	Asp	Tyr	Leu	Pro
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Ala	Ala	Val	Ser	Asn	Pro	Met	Pro	Gly	Asn	Trp	Glu	Leu	Val	Trp	Thr
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Gly	Phe	Asn	Phe	Ala	Pro	Leu	Tyr	Glu	Ser	Gly	Phe	Leu	Val	Arg	Ile
865						870					875				880
Tyr	Gly	Val	Glu	Ile	Thr	Pro	Ser	Val	Trp	Tyr	Ile	Asn	Arg	Thr	Tyr
				885					890					895	
Leu	Asp	Thr	Asn	Thr	Glu	Phe	Ser	Ile	Glu	Phe	Asn	Ile	Thr	Asn	Ile
			900					905					910		
Tyr	Ala	Pro	Ile	Asn	Ala	Thr	Leu	Ile	Pro	Ile	Gly	Leu	Gly	Thr	Tyr
		915					920					925			
Asn	Ala	Ser	Val	Glu	Ser	Val	Gly	Asp	Gly	Glu	Phe	Phe	Ile	Lys	Gly
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Ile	Glu	Val	Pro	Glu	Gly	Thr	Ala	Glu	Leu	Lys	Ile	Arg	Ile	Gly	Asn
945						950					955				960
Pro	Ser	Val	Pro	Asn	Ser	Asp	Leu	Asp	Leu	Tyr	Leu	Tyr	Asp	Ser	Lys
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Val Val Glu Tyr Pro Lys Pro Gly Val Tyr Ser Ile Val Val His Gly
 995 1000 1005

Tyr Ser Val Arg Asp Glu Asn Gly Asn Pro Thr Thr Thr Thr Phe
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Asp Leu Val Val Gln Met Thr Leu Asp Asn Gly Asn Ile Lys Leu
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Asp Lys Asp Ser Ile Ile Leu Gly Ser Asn Glu Ser Val Val Val
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Thr Ala Asn Ile Thr Ile Asp Arg Asp His Pro Thr Gly Val Tyr
 1055 1060 1065

Ser Gly Ile Ile Glu Ile Arg Asp Asn Glu Val Tyr Gln Asp Thr
 1070 1075 1080

Asn Thr Ser Ile Ala Lys Ile Pro Ile Thr Leu Val Ile Asp Lys
 1085 1090 1095

Ala Asp Phe Ala Val Gly Leu Thr Pro Ala Glu Gly Val Leu Gly
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Glu Ala Arg Asn Tyr Thr Leu Ile Val Lys His Ala Leu Thr Leu
 1115 1120 1125

Glu Pro Val Pro Asn Ala Thr Val Ile Ile Gly Asn Tyr Thr Tyr
 1130 1135 1140

Leu Thr Asp Glu Asn Gly Thr Val Thr Phe Thr Tyr Ala Pro Thr
 1145 1150 1155

Lys Leu Gly Ser Asp Glu Ile Thr Val Ile Val Lys Lys Glu Asn
 1160 1165 1170

Phe Asn Thr Leu Glu Lys Thr Phe Gln Ile Thr Val Ser Glu Pro
 1175 1180 1185

Glu Ile Thr Glu Glu Asp Ile Asn Glu Pro Lys Leu Ala Met Ser

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Ser Pro Glu Ala Asn Ala Thr Ile Val Ser Val Glu Met Glu Ser		
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Glu Gly Gly Val Lys Lys Thr Val Thr Val Glu Ile Thr Ile Asn		
1220	1225	1230
Gly Thr Ala Asn Glu Thr Ala Thr Ile Val Val Pro Val Pro Lys		
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Lys Ala Glu Asn Ile Glu Val Ser Gly Asp His Val Ile Ser Tyr		
1250	1255	1260
Ser Ile Glu Glu Gly Glu Tyr Ala Lys Tyr Val Ile Ile Thr Val		
1265	1270	1275
Lys Phe Ala Ser Pro Val Thr Val Thr Val Thr Tyr Thr Ile Tyr		
1280	1285	1290
Ala Gly Pro Arg Val Ser Ile Leu Thr Leu Asn Phe Leu Gly Tyr		
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Ser Trp Tyr Arg Leu Tyr Ser Gln Lys Phe Asp Glu Leu Tyr Gln		
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Lys Ala Leu Glu Leu Gly Val Asp Asn Glu Thr Leu Ala Leu Ala		
1325	1330	1335
Leu Ser Tyr His Glu Lys Ala Lys Glu Tyr Tyr Glu Lys Ala Leu		
1340	1345	1350
Glu Leu Ser Glu Gly Asn Ile Ile Gln Tyr Leu Gly Asp Ile Arg		
1355	1360	1365
Leu Leu Pro Pro Leu Arg Gln Ala Tyr Ile Asn Glu Met Lys Ala		
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Val Lys Ile Leu Glu Lys Ala Ile Glu Glu Leu Glu Gly Glu Glu		
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<222> (130)..(130)

<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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ggc aag gtc ata ggc tgg tac gac gcc gtc aac ggc agg tcg acc ccc 97
Gly Lys Val Ile Gly Trp Tyr Asp Ala Val Asn Gly Arg Ser Thr Pro
20 25 30

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Tyr Asp Asp Gln Gly His Gly Thr His Val Ala Gly Thr Val Ala Gly
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<211> 564

<212> DNA

<213> Thermococcus celer

<400> 14

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Asn Ser Gln Tyr Ile Gly Val Ala Pro Gly Ala Lys Leu Val Gly Val
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aag gtt ctc ggt gcc gac ggt tcg gga agc gtc tcc acc atc atc gcg 144
Lys Val Leu Gly Ala Asp Gly Ser Gly Ser Val Ser Thr Ile Ile Ala
35 40 45

ggt gtt gac tgg gtc gtc cag aac aag gat aag tac ggg ata agg gtc 192
Gly Val Asp Trp Val Val Gln Asn Lys Asp Lys Tyr Gly Ile Arg Val
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atc aac ctc tcc ctc ggc tcc tcc cag agc tcc gac gga gcc gac tcc 240
Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser Ser Asp Gly Ala Asp Ser
65 70 75 80

ctc agt cag gcc gtc aac aac gcc tgg gac gcc ggt ata gta gtc tgc 288
Leu Ser Gln Ala Val Asn Asn Ala Trp Asp Ala Gly Ile Val Val Cys
85 90 95

gtc gcc gcc ggc aac agc ggg ccg aac acc tac acc gtc ggc tca ccc 336
Val Ala Ala Gly Asn Ser Gly Pro Asn Thr Tyr Thr Val Gly Ser Pro
100 105 110

gcc gcc gcg agc aag gtc ata acc gtc ggt gca gtt gac agc aac gac 384
Ala Ala Ala Ser Lys Val Ile Thr Val Gly Ala Val Asp Ser Asn Asp

115	120	125	
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Asn Ile Ala Ser Phe Ser Ser Arg Gly Pro Thr Ala Asp Gly Arg Leu			
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aag ccg gaa gtc gtc gcc ccc ggc gtt gac atc ata gcc ccg cgc gcc			480
Lys Pro Glu Val Val Ala Pro Gly Val Asp Ile Ile Ala Pro Arg Ala			
145	150	155	160
agc gga acc agc atg ggc acc ccg ata aac gac tac tac acc aag gcc			528
Ser Gly Thr Ser Met Gly Thr Pro Ile Asn Asp Tyr Tyr Thr Lys Ala			
165	170	175	
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 <212> DNA
 <213> Thermococcus celer

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<210> 17
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33

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<220>
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<211> 22	
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<223> Synthetic

<400> 24
ctgttccagc tacgtgagtt cc 22

<210> 25
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
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<400> 25
ctgttccagc tacatgagtt cc 22

<210> 26
<211> 507
<212> DNA
<213> Pyrococcus furiosus

<400> 26
a cta gtc atc tca ggt tta aca ggg ggt aaa gct aag ctt tca ggt gtt 49
Leu Val Ile Ser Gly Leu Thr Gly Gly Lys Ala Lys Leu Ser Gly Val
1 5 10 15

agg ttt atc cag gaa gac tac aaa gtt aca gtt tca gca gaa tta gaa 97
Arg Phe Ile Gln Glu Asp Tyr Lys Val Thr Val Ser Ala Glu Leu Glu
20 25 30

gga ctg gat gag tct gca gct caa gtt atg gca act tac gtt tgg aac 145
Gly Leu Asp Glu Ser Ala Ala Gln Val Met Ala Thr Tyr Val Trp Asn
35 40 45

ttg gga tat gat ggt tct gga atc aca ata gga ata att gac act gga 193
Leu Gly Tyr Asp Gly Ser Gly Ile Thr Ile Gly Ile Ile Asp Thr Gly
50 55 60

att gac gct tct cat cca gat ctc caa gga aaa gta att ggg tgg gta 241
Ile Asp Ala Ser His Pro Asp Leu Gln Gly Lys Val Ile Gly Trp Val
65 70 75 80

gat ttt gtc aat ggt agg agt tat cca tac gat gac cat gga cat gga 289
Asp Phe Val Asn Gly Arg Ser Tyr Pro Tyr Asp Asp His Gly His Gly
85 90 95

act cat gta gct tca ata gca gct ggt act gga gca gca agt aat ggc 337
Thr His Val Ala Ser Ile Ala Ala Gly Thr Gly Ala Ala Ser Asn Gly
100 105 110

aag tac aag gga atg gct cca gga gct aag ctg gcg gga att aag gtt 385
Lys Tyr Lys Gly Met Ala Pro Gly Ala Lys Leu Ala Gly Ile Lys Val
115 120 125

cta ggt gcc gat ggt tct gga agc ata tct act ata att aag gga gtt 433
Leu Gly Ala Asp Gly Ser Gly Ser Ile Ser Thr Ile Ile Lys Gly Val

130	135	140	
gag tgg gcc gtt gat aac aaa gat aag tac gga att aag gtc att aat			481
Glu Trp Ala Val Asp Asn Lys Asp Lys Tyr Gly Ile Lys Val Ile Asn			
145	150	155	160
ctt tct ctt ggt tca agc cag agc tc			507
Leu Ser Leu Gly Ser Ser Gln Ser			
165	-		
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<211> 30			
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<223> Synthetic			
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<223> Synthetic			
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<211> 30			
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			Met
			1
aag ggg ctg aaa gct ctc ata tta gtg att tta gtt cta ggt ttg gta			106
Lys Gly Leu Lys Ala Leu Ile Leu Val Ile Leu Val Leu Gly Leu Val			

5	10	15	
gta ggg agc gta gcg gca gct cca gag aag aaa gtt gtt caa gta aga Val Gly Ser Val Ala Ala Ala Pro Glu Lys Lys Val Val Gln Val Arg 20 25 30			154
aat gtt gag aag aac tat ggt ctg cta acg cca gga ctg ttc aga aaa Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg Lys 35 40 45			202
att ccc aaa ttg gat cct aac gag gga atc agc aca gta att gta ttt Ile Pro Lys Leu Asp Pro Asn Glu Gly Ile Ser Thr Val Ile Val Phe 50 55 60 65			250
gtt aac cat agg gga aaa gaa att gca gta aga gtt ctt gag tta atg Val Asn His Arg Gly Lys Glu Ile Ala Val Arg Val Leu Glu Leu Met 70 75 80			298
ggt gcc caa gtt agg tat gtg tac cat att ata ccc cca ata gct gcc Gly Ala Gln Val Arg Tyr Val Tyr His Ile Ile Pro Pro Ile Ala Ala 85 90 95			346
gat ctt aag gtt aga gac tta cta gtc atc tca ggt tta aca ggg ggt Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly Gly 100 105 110			394
gaa act aag ctt tca ggt gtt agg t Glu Thr Lys Leu Ser Gly Val Arg 115 120			419

<210> 31
 <211> 180
 <212> DNA
 <213> Pyrococcus furiosus

<400> 31 gctctagact ctgggaggag tagttatact tgatgaagcc tattctgagt tttcgggaaa	60
aagcttcata ccaaaaatca gtgagtatga aaatttagta attctaagga cgttttcaaa	120
ggcgtttgga cttgctggaa ttagatgtgg atatatgata gcaaatgaaa agattataga	180

<210> 32
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 32 agagggatcc atgaaggggc tgaaagct	28
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<210> 33
 <211> 30

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 33
 agagggcatgc gctctagact ctgggagagt

30

<210> 34
 <211> 1962
 <212> DNA
 <213> *Pyrococcus furiosus*

<400> 34
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 ctgctaacgc caggactggt cagaaaaatt caaaaattga atcctaacga ggaaatcagc 180
 acagtaattg tatttgaaaa ccatagggaa aaagaaattg cagtaagagt tcttgagtta 240
 atgggtgcaa aagttaggta tgtgtaccat attatacccg caatagctgc cgatcttaag 300
 gttagagact tactagtcac ctccaggttta acagggggta aagctaagct ttcagggtgtt 360
 aggtttatcc aggaagacta caaagttaca gtttcagcag aattagaagg actggatgag 420
 tctgcagctc aagttatggc aacttacgtt tggaacttgg gatatgatgg ttctggaatc 480
 acaataggaa taattgacac tggaaattgac gcttctcatc cagatctcca aggaaaagta 540
 attgggtggg tagattttgt caatggtagg agttatccat acgatgacca tggacatgga 600
 actcatgtag cttcaatagc agctgggtact ggagcagcaa gtaatggcaa gtacaagggg 660
 atggctccag gagctaagct ggcgggaatt aaggttctag gtgccgatgg ttctggaagc 720
 atatctacta taattaaggg agttgagtgg gccgttgata acaaagataa gtacggaatt 780
 aaggctatta atctttctct tggttcaagc cagagctcag atggtactga cgctctaagt 840
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 ggacctaaaca agtatacaat cggttctcca gcagctgcaa gcaaagttat tacagttgga 960
 gccgttgaca agtatgatgt tataacaagc ttctcaagca gagggccaac tgcagacggc 1020
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 gacaaagtaa aaacagccct catagaaact gctgatatcg taaagccaga tgaaatagcc 1260

gatatagcct acggtgcagg taggggtaat gcatacaagg ctataaacta cgataactat 1320
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attagcggag cttcgttcgt aactgccaca ttatactggg acaatgcaa tagcgacctt 1440
gatctttacc tctacgatcc caatggaaac caggttgact actcttacac cgctactat 1500
ggattcgaaa aggttggtta ttacaaccca actgatggaa catggacaat taaggttgta 1560
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cctggaagtt caccatctcc acaaccagaa ccaacagtag acgcaaagac gttccaagga 1680
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tacgatccta accagaagct tgtagataga tcggagagtc ccaacagcta cgaacacgta 1860
gaatacttaa cccccgcccc aggaacctgg tacttcctag tatatgccta ctacacttac 1920
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<210> 35
<211> 654
<212> PRT
<213> *Pyrococcus furiosus*

<400> 35

Met Lys Gly Leu Lys Ala Leu Ile Leu Val Ile Leu Val Leu Gly Leu
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Val Val Gly Ser Val Ala Ala Ala Pro Glu Lys Lys Val Glu Gln Val
20 25 30

Arg Asn Val Glu Lys Asn Tyr Gly Leu Leu Thr Pro Gly Leu Phe Arg
35 40 45

Lys Ile Gln Lys Leu Asn Pro Asn Glu Glu Ile Ser Thr Val Ile Val
50 55 60

Phe Glu Asn His Arg Glu Lys Glu Ile Ala Val Arg Val Leu Glu Leu
65 70 75 80

Met Gly Ala Lys Val Arg Tyr Val Tyr His Ile Ile Pro Ala Ile Ala
85 90 95

Ala Asp Leu Lys Val Arg Asp Leu Leu Val Ile Ser Gly Leu Thr Gly
100 105 110

Gly Lys Ala Lys Leu Ser Gly Val Arg Phe Ile Gln Glu Asp Tyr Lys
 115 120 125

Val Thr Val Ser Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
 130 135 140

Val Met Ala Thr Tyr Val Trp Asn Leu Gly Tyr Asp Gly Ser Gly Ile
 145 150 155 160

Thr Ile Gly Ile Ile Asp Thr Gly Ile Asp Ala Ser His Pro Asp Leu
 165 170 175

Gln Gly Lys Val Ile Gly Trp Val Asp Phe Val Asn Gly Arg Ser Tyr
 180 185 190

Pro Tyr Asp Asp His Gly His Gly Thr His Val Ala Ser Ile Ala Ala
 195 200 205

Gly Thr Gly Ala Ala Ser Asn Gly Lys Tyr Lys Gly Met Ala Pro Gly
 210 215 220

Ala Lys Leu Ala Gly Ile Lys Val Leu Gly Ala Asp Gly Ser Gly Ser
 225 230 235 240

Ile Ser Thr Ile Ile Lys Gly Val Glu Trp Ala Val Asp Asn Lys Asp
 245 250 255

Lys Tyr Gly Ile Lys Val Ile Asn Leu Ser Leu Gly Ser Ser Gln Ser
 260 265 270

Ser Asp Gly Thr Asp Ala Leu Ser Gln Ala Val Asn Ala Ala Trp Asp
 275 280 285

Ala Gly Leu Val Val Val Val Ala Ala Gly Asn Ser Gly Pro Asn Lys
 290 295 300

Tyr Thr Ile Gly Ser Pro Ala Ala Ala Ser Lys Val Ile Thr Val Gly
 305 310 315 320

Ala Val Asp Lys Tyr Asp Val Ile Thr Ser Phe Ser Ser Arg Gly Pro
 325 330 335

Thr Ala Asp Gly Arg Leu Lys Pro Glu Val Val Ala Pro Gly Asn Trp
 340 345 350

Ile Ile Ala Ala Arg Ala Ser Gly Thr Ser Met Gly Gln Pro Ile Asn
 355 360 365

Asp Tyr Tyr Thr Ala Ala Pro Gly Thr Ser Met Ala Thr Pro His Val
 370 375 380

Ala Gly Ile Ala Ala Leu Leu Leu Gln Ala His Pro Ser Trp Thr Pro
 385 390 395 400

Asp Lys Val Lys Thr Ala Leu Ile Glu Thr Ala Asp Ile Val Lys Pro
 405 410 415

Asp Glu Ile Ala Asp Ile Ala Tyr Gly Ala Gly Arg Val Asn Ala Tyr
 420 425 430

Lys Ala Ile Asn Tyr Asp Asn Tyr Ala Lys Leu Val Phe Thr Gly Tyr
 435 440 445

Val Ala Asn Lys Gly Ser Gln Thr His Gln Phe Val Ile Ser Gly Ala
 450 455 460

Ser Phe Val Thr Ala Thr Leu Tyr Trp Asp Asn Ala Asn Ser Asp Leu
 465 470 475 480

Asp Leu Tyr Leu Tyr Asp Pro Asn Gly Asn Gln Val Asp Tyr Ser Tyr
 485 490 495

Thr Ala Tyr Tyr Gly Phe Glu Lys Val Gly Tyr Tyr Asn Pro Thr Asp
 500 505 510

Gly Thr Trp Thr Ile Lys Val Val Ser Tyr Ser Gly Ser Ala Asn Tyr
 515 520 525

Gln Val Asp Val Val Ser Asp Gly Ser Leu Ser Gln Pro Gly Ser Ser
 530 535 540

Pro Ser Pro Gln Pro Glu Pro Thr Val Asp Ala Lys Thr Phe Gln Gly
 545 550 555 560

Ser Asp His Tyr Tyr Tyr Asp Arg Ser Asp Thr Phe Thr Met Thr Val
 565 570 575

Asn Ser Gly Ala Thr Lys Ile Thr Gly Asp Leu Val Phe Asp Thr Ser
 580 585 590

Tyr His Asp Leu Asp Leu Tyr Leu Tyr Asp Pro Asn Gln Lys Leu Val
 595 600 605

Asp Arg Ser Glu Ser Pro Asn Ser Tyr Glu His Val Glu Tyr Leu Thr
 610 615 620

Pro Ala Pro Gly Thr Trp Tyr Phe Leu Val Tyr Ala Tyr Tyr Thr Tyr
 625 630 635 640

Gly Trp Ala Tyr Tyr Glu Leu Thr Ala Lys Val Tyr Tyr Gly
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<210> 36
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 36
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<210> 37
 <211> 20
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<220>
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<400> 37
 tgtactgctg gatccggcag 20

<210> 38
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 <212> DNA
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<220>
 <223> Synthetic

<400> 38
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atatctcccg cgggtaaggt 80

<210> 39
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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 39
agaggcatgc gtatccatca gatttttgag 30

<210> 40
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<213> Artificial Sequence

<220>
<223> Synthetic

<400> 40
agtgaacgga tacttggaac 20

<210> 41
<211> 20
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<220>
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<400> 41
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<210> 42
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<212> PRT
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<400> 42

Ala Glu Leu Glu Gly Leu Asp Glu Ser Ala Ala Gln
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<210> 43
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<220>

<221> misc_feature
 <222> (1)..(1)
 <223> Leu is modified by a succinyl group.

 <220>
 <221> misc_feature
 <222> (4)..(4)
 <223> Tyr is modified by a 7-amino-4-methylcoumarin group.

 <400> 43

Leu Leu Val Tyr
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<210> 44
 <211> 4
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Ala is modified by a succinyl group.

<220>
 <221> misc_feature
 <222> (4)..(4)
 <223> Phe is modified by a p-nitroaniline group.

<400> 44

Ala Ala Pro Phe
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<210> 45
 <211> 382
 <212> PRT
 <213> subtilisin

<400> 45

Met Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu
 1 5 10 15

Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly
 20 25 30

Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met
 35 40 45

Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly
 50 55 60

Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr
 65 70 75 80

Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala
 85 90 95

Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro
 100 105 110

Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr
 115 120 125

Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser
 130 135 140

Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser
 145 150 155 160

Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala
 165 170 175

Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala
 180 185 190

Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu Gly Ala Asp Gly Ser
 195 200 205

Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn
 210 215 220

Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala
 225 230 235 240

Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala Ser Gly Val Val Val
 245 250 255

Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Ser Thr Val
 260 265 270

Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp

275

280

285

Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu Asp
290 295 300

Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys
305 310 315 320

Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val Ala Gly
325 330 335

B6 Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr Gln
340 345 350

Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys Leu Gly Asp Ser Phe
355 360 365

Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
370 375 380
